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## N-TERMINAL AMINOACID SEQUENCES

	MINAL AIVIIN	OACID SEQ	UENCES
Position 01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39	GLN SER SER ASP THR VAL ASP GLN	ALAR	C LEU ALA VALA PRA ASIN GLER ASIN GLER VALA ASIN SER VALA ASIN ASIN ASIN ASIN ASIN ASIN ASIN ASI

Figure 1a

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# PEPTIDE AMINOACID SEQUENCES

		Α	В	С	D	E
P	ositior	<b>1</b>				
	01	GLN	(TRP)*	MET	ALA	VAL
	02	**	SER	MET	SER	VAL
	03	GLN	PHE	GLN	SER	ASP
	04	ALA	ASP	CYS	ALA	
	05	GLU	THR	GLN	GLU	ARG
	06	GLN	ILE	ALA	LYS	PHE
	07	GLU	SER	GLU	GLY	PRO
	08	PRO	THR	GLN	TYR	TYR
	09	LEU	SER	GLU	ASP	THR
	10	VAL	THR	PRO	LEU	GLY
	11	(ARG)	VAL	LEU	VAL	
	12	VAL	ASP	VAL	VAL	ALA
	13	LEU	THR	ARG		
	14	VAL	LYS	VAL		
	15	ASN	LEU	LEU		
	16	(ASP)	SER	VAL		
	17	(ARG)	PRO	ASN		
	18	(VAL)	PHE	ASP		•
	19	VAL	(CYS)	ARG		
	20	PRO	(ASP)			
	21		LEU			
	22		PHE			
	23	•	THR			

Figure 1b

Application No.: To be Assigned Docket No.: 24615-20026.03

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PCT/NL90/00140

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# N-TERMINUS 100KD PROTEIN

Position	, .	•
01		VAL
02	4	VAL
03	-	ASP
04	a a	GLU
05	,	ARG
06		PHE
07	, ,	PRO
08	~ '	TYR
09	•	THR
. 10	-	GLY

Figure 1c

Application No.: To be Assigned Docket No.: 24615-20026.03

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Figure 2a (sheet 1 of 2)

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Peptide B:		-he-	Ser-	음	Thr-	Ser	土高	一一	-Arg-	(GB)	등	-Tyr-	-Ala-	Pro-	Phe	-Phe-	(Asp)	Ten	-Ala
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Figure 2a (sheet 2 of 2

Sheet 6 of 36

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·	Ser/A	rcg/c	4	<b>-</b>	ပ	AGT AGG	ပ
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3'- GTC, CGC, CTC, GTC, GGG, GAG, CA-5 ⋖ G <del>1</del>0

-GAT/ACG/CGG-(CGG/GTG)-GTG-CCG-(CCG)-ATG-GGG 4 Figure 2b (sheet 1 of

AB1295:

Inventor: Robert VAN GORCOM et al.
Application No.: To be Assigned
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(TGG)-TCG-TTT-GAT-ACG-ATA-TCG-ACG-GTG-GAT-ACG-AAG-CTG-TCG-CCG-TTT-A A A A A A A A A A A A C A A A A C C C C A A A A A C		-									
A C C C C S'-AAG.		(TGG)-TCG-TTT-GA	AT-ACG-ATA-TCG	-ACG -TCG-A	CG-GTG	-GAT-	CG-AA	G-CTG-	-TCG-(	CG-T	- <u></u> -L
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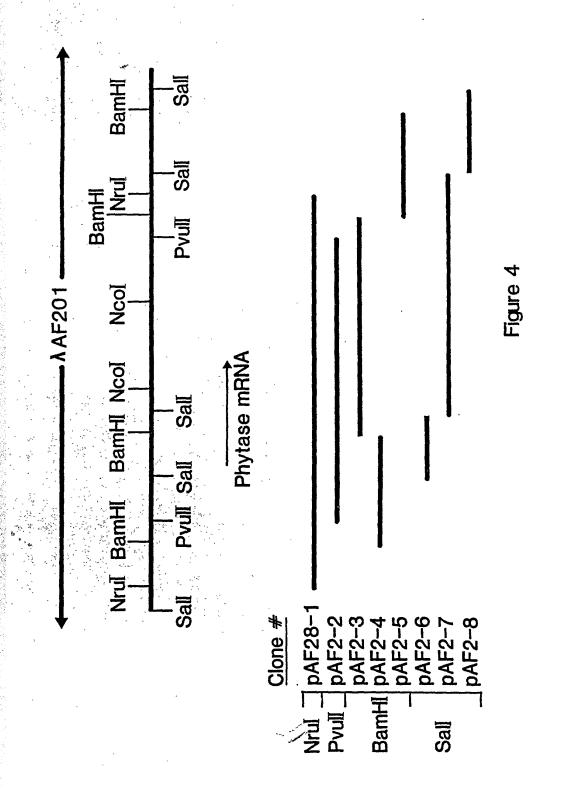
(ACG). (CTG). GAG. AAG. TGC. (TGC). (CTG). (CTC). (ACG).(TAG). (T)-5'

Figure 2b (sheet 2 of

Sheet 8 of 36

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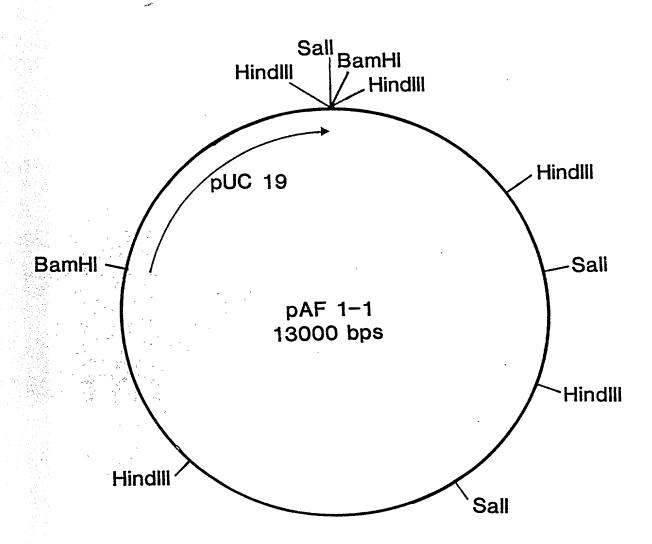


Figure 5

PCT/NL90/00140

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Figure 6 (sheet 1 of 7)

•			
GTCGACTTCCCGTCCTATTCGGCCTCGTCCGCTGAAGATCCATCC			
TTGCACGTGGGCCACCTTTGTGAGCTTCTAACCTGAACTGGTAGAGTATC	100		
ACACACCATGCCAAGGTGGGATGAAGGGGTTATATGAGACCGTCCGGTCC			
GGCGCGATGGCCGTAGCTGCCACTCGCTGCTGCAAGAAATTACTTCTC	200		
ATAGGCATCATGGGCGTCTCTGCTGTTCTACTTCCTTTGTATCTCCTGTC translation start			
TGGGTATGCTAAGCACCACAATCAAAGTCTAATAAGGACCCTCCCT	300		
AGGGCCCCTGAAGCTCGGACTGTGTGGGACTACTGATCGCTGACTATCTG			
TGCAGAGTCACCTCCGGACTGGCAGTCCCCGCCTCGAGAAATCAATC	400		
TTGCGATACGGTCGATCAGGGGTATCAATGCTTCTCCGAGACTTCGCATC	•	414	
TTTGGGGTCAATACGCACCGTTCTTCTCTCTGGCAAACGAATCGGTCATC	500	90	
TCCCCTGAGGTGCCCGCCGGATGCAGAGTCACTTTCGCTCAGGTCCTCTC			
CCGTCATGGAGCGCGGTATCCGACCGACTCCAAGGGCAAGAAATACTCCG	600		
CTCTCATTGAGGAGATCCAGCAGAACGCGACCACCTTTGACGGAAAATAT			
GCCTTCCTGAAGACATACAACTACAGCTTGGGTGCAGATGACCTGACTCC	700		
CTTCGGAGAACAGGAGCTAGTCAACTCCGGCATCAAGTTCTACCAGCGGT			
ACGAATCGCTCACAAGGAACATCGTTCCATTCATCCGATCCTCTGGCTCC	800		
AGCCGCGTGATCGCCTCCGGCAAGAAATTCATCGAGGGCTTCCAGAGCAC			414 400
CAAGCTGAAGGATCCTCGTGCCCAGCCCGGCCAATCGTCGCCCAAGATCGBamhi	900		8 = 4
ACGTGGTCATTTCCGAGGCCAGCTCATCCAACAACACTCTCGACCCAGGC	968	<b>.</b>	17
ACCTGCACTGTCTTCGAAGACAGCGAATTGGCCGATACCGTCGAAGCCAA	1000		

Inventor: Robert VAN GORCOM et al. Application No.: To be Assigned

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Figure 6 (sheet 2 of 7)

TTTCACCGCCACGTTCGTCCCCTCCATTCGTCAACGTCTGGAGAACGACC TGCTCCTTCGACACCATCTCCACCAGCACCGTCGACACCAAGCTGTCCCC CTTCTGTGACCTGTTCACCCATGACGAATGGATCAACTACGACTACCTCC AGTCCTTGAAAAAGTATTACGGCCATGGTGCAGGTAACCCGCTCGGCCCG ACCCAGGGCGTCGCTACGCTAACGAGCTCATCGCCCGTCTGACCCACTC GCCTGTCCACGATGACACCAGTTCCAACCACACTTTGGACTCGAGCCCGG CTACCTTTCCGCTCAACTCTACTCTCTACGCGGACTTTTCGCATGACAAC GGCATCATCTCCATTCTCTTTGCTTTAGGTCTGTACAACGGCACTAAGCC GCTATCTACCACGACCGTGGAGAATATCACCCAGACAGATGGATTCTCGT CTGCTTGGACGGTTCCGTTTGCTTCGCGTTTGTACGTCGAGATGATGCAG TGTCAGGCGGAGCAGGAGCCGCTGGTCCGTGTCTTGGTTAATGATCGCGT 1600 TGTCCCGCTGCATGGGTGTCCGGTTGATGCTTTGGGGAGATGTACCCGGG ATAGCTTTGTGAGGGGGTTGAGCTTTGCTAGATCTGGGGGTGATTGGGCG 1700 translation stop CATATCATTAGCACTTCAGGTATGTATTATCGAAGATGTATATCGAAAGG 1800 ATCAATGGTGACTGTCACTGGTTATCTGAATATCCCTCTATACCTCGTCC CACAACCAATCACCCTTTAAACAATCACACTCAACGCACAGCGTACA AACGAACAAACGCACAAAGAATATTTTACACTCCTCCCCAACGCAATACC 2000

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### Figure 6 (sheet 3 of 7)

ATCCCTACCCTCAAGTCCACCCATCCTATAATCAATCCCTACTTACT	
TTCTCCCCCTCCCCTCACCCTTCCCAGAACTCACCCCCGAAGTAGTAAT	2100
AGTAGTAGTAGAAGAAGCAGACGACCTCTCCACCAATCTCTTCGGCCTCT	
TATCCCCATACGCTACACAAAACCCCCACCCCGTTAGCATGCACTCAGAA	2200
AATAATCAAAAATAACTAAGAAGGAAAAAAAAAAGAAGAAGAAAGGTTACAT	
ACTCCTCTCATACAAACTCCAAGACGTATACATCAAGATGGGCAATCCCA	2300
CCATTACTGATATCCATCTATGAACCCATTCCCATCCCA	
TACTTTACTTAGAAGAAGAAAAGGGAAGGGAAAGAAGTGGATGG	2400
GATTGAGTTAGTGCTCACCGTCTCGCAGCAAGTTTATATTCTTTTGTTTG	
GCGGATATCTTTCACTGCTCCTGCTGGACGTTGTCACGGGGTGGTAGTGG	2500
TTGGCGGTGGTGAGGGTCCATGATCACTCTTGGTTTGGGGGGGTTGTTGTT	
GTCGTTGTTGTTGTTGGGTGGGCATTTTCTTTCTTCACTTGGGGAT	2600
TATTATTTGGAATTGGTTAGTTTGAGTGAGTGGGTAATATTGAATGGGTG	
ATTATTGGGAATGAAGTAGATTTGGCTATGAATGGTTGATGGGATGGAAT	2700
GAATGGATGGATGAATAGATGGAGGCGGAAAAGTCAGGTGGTTTGAGGTT	
CGGATTATTATCTTTGTGCCTGAGGCATCACTCTCCATCTATGTTGTTCT	2800
TTCTATACCGATCTACCAGAGCTAAGTTGACTGATTCTACCACAGTGCAC	
AATAAGTATGTACTTATTTCATTTAGAGTATTTAGATTAACCCGCTGTGC	2900
TATTTGCCGTAGCTTTCCACCCAATTTCGAAGTTCGAAGAATTAAAACTC	
ATCCTACAGTACAGAATAGAAGTAAAAGGAGAAGAGAAAAACAAGATAAT	3000

Title: CLONING AND EXPRESSION OF MICROBIAL PHYTASE Inventor: Robert VAN GORCOM et al.
Application No.: To be Assigned
Docket No.: 24615-20026.0314/36

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#### Figure 6 (sheet 4 of 7)

ACAACCAGTCCAGGTCCATTCTAGATCTCGAATGACCACCAAATAAGAAA	
GCAACAAGCAAGCAAAGCATAAGTCTAAATGAACGCCAATAACTTC	3100
ATCGCCTGCCTTTGAAACTGAACGCTATGCACGAATGGCTCGAAATGATT	
CCCTTAACTCCGTAGTATTGAGAGTGAGAGAAAAAAAAAA	3200
AAAAGCTGACCATGGGAAAGAAGCATGATCAGTCGGGAATGGATCTGCGG	
GTTGAGATAGAGTTGCCTCGCAGATCCGGTGACAAGATAAGAGAA	3300
TTGGGAGATGTGATCAGCCACTGTAACTTCATCAAGCATCGACATTCAAC	
GGTCGGGTCTGCGGGTTGAGATGCAAGTTGAGATGCCACGCAGACCCGAA	3400
CAGAGTGAGAGATGTGAGACTTTTGAACCACTGTGACTTCATCAAGCATC	
AAAACACACTCCATGGTCAATCGGTTAGGGTGTGAGGGTTGATATGCCAG	3500
GTTCGATGCCACGCAGACCGACCGACTGAGAAATATGAAAAGTTGGAC	
AGCCACTTCATCATCAAGCGTAAAACCCCAATCAATGGTAAATCGAA	3600
AACGAATCTGCGGGCTGATGTGGAAATGAGACGAATGCCTCGCAGATTCG	
AAGACACGTAAATCGAGATGAACAATCACTTTAACTTCATCAAAGCCTTA	3700
AATCACCCAATGGCCAGTCTATTCGGGTCTGCGGGTTGAGGTTCCTGTTG	
AGATGCCACGCAGACTGCGAACATGCGATGCATTATAAGTTGGACGAGTG	3800
TAGACTGACCATTGATAACCGAGATAAACAATCACTTCAACTTCAACA	
GCCTTAAATCACTCAATGGCCAGTCTGTTTGCGGTCTGCGGGCTGATACC	3900
CAAGTTGCGATGCCACGCAGACTGCAAACATTGATCGAGAGACGAGAAAA	
ACAACGCACTTTAACTTCAACAAAAGCCTTTCAATCAGTCAATGGCCAGT	4000

Title: CLONING AND EXPRESSION OF MICROBIAL PHYTASE Inventor: Robert VAN GORCOM et al.

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### Figure 6 (sheet 5 of 7)

CTGTTCGCGGTCTGCGGGCTGATATGCGAGTTGAGGTGCCTCGCAGACCG	
CGAACATGCGATGTAATTTCTTAGTTAGACGAGTGCCTGGCCATTGAGAA	4100
ACGAGAGAAACAACCACTTTAACTTCATGAAAGCCTTGAACTACTCAATG	
ACCCGTCTGTTGGCGGTCTGCGGGCTGATATTCGAGTTGAGATGCCACGC	4200
AGACCGCCAACATGCGATGTATCATGTAAGTTAGATGAGTGACTGGCCAT	
TGAGAAACGAGAAACAACCACACTTCATGAGAGCCTTAAATTATTCAA	4300
TGACCAGTCTGTTCACGGTCTGCGGGTTGGTATGCGAGTCGAGGTGCCTC	
GCAGACCGCGAACATGCGATGTTTTCGATGGACGAGTGAAGCCTGACGAT	4400
CGAGAACTATCTCAGTTGGGTTGGCCATTCGGCTGGCCGTTGGGTTTAGT	
ATTAGGATCGTCAGGTTTGTCCGATGGAACGTTCCGTTTGCGTGCG	4500
CGCGACGAGCCCTCTCCTCGGCGTGATTCTGAAATTCTGCAATCAGGGCA	
GCCGCAGCACGGGACGTCCTCCAGGAGCTGTGTTGAAGTTTCGG	4600
GGTGGCGGTCCAGAAGGGGGAGTTACATTAAAAGCCTCATAGATGTCTTT	
GGGTGGTTCCGGGGGGCCCATCGCAAGATCTTCTGGAGTTGTGCGTCTGA	4700
TCATCTCTTGAGTGTAATTGCGACGCAGACCGAGCTTCAGGATTTTGGAA	
GGGCTGGATCGCTCCTGACTCTTTCCCTCAGCGGGCTTCGTCTCGGC	4800
AGTCTTCATTTCGGCGGGCTGATCTTCCATCTCAGAATGGGATCGCTTTC	
TGGTCGCTGCACCCGCTCCCTTCAAGGTCAGCTTGATGCGCAGCGTC	4900
TTGGGCGCTCAGCTGGTGGAGTTGGTTCCGGCTCTGGCTCCCTCC	
TCGCTTGGGCACTTGAGTAGTCTCTGAGGCTTCGCCGCGCGCG	5000

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Figure 6 (sheet 6 of 7)

TTCGGGGCGGTTTCATCGTGCTGAGCGATCAAGGTTTGGATGTAGGCAGC CGGCATCATTCGATCAACGGCAATTCCTCTCTTGCGGGCCTCCTCCCGAG CCTTGATTGTCGCCTTGACCTCGTCCACGTTTTCGAAGAAGAAAGGCATC 5200 TTGTTATCCTGAGGCAAGTTGCGCTCTCCCATGCGTGGGGATATCCGAAG ATGCGGTCCTTCTCGAACTGTTCATGAGACTTCAGACGAATTGGAGGCTG 5300 GGGGAGCAATTTGTCTCCGTAGGTGTTGTTAGGGCGGAACCAAGAATAGC CTTCGCCTACAACGACAAGCTCTTCGCCAAATTTATTTTTTTGGCCTGTA 5400 AAAACGAACCCATCCTCGTCAGTCCACCGGTGCGTCTCGGACGTAGAGAT TGGCTTACTTATTCCCTCAACGCCGATCTCTGCCTGGGGCTGCGCTTCGG 5500 ATGCGGCCTCGGTCACGGCTCCGCCTCGGACTGCACCGCTGGAGTTTCGG TCTTCTTCTCCTGCTTCTCCAGGTACTCCTTGCGTAACTCTTCGATCAGC CTCGGCTTCCGATGACTGCTCAAATTCTGGAGCAACAGCTGCCGCGGCCA GGTCAAGCAGCGGTTTGCTAAAACTGCCCATTTTCCATCGACACCTGCC 5700 ACGCGTCTTCTTGACTGCTGCCTTGCCCTTTACTTCCTTGAGAGCAGACT 5800 CTGGCTTAGATGATGGTGCACGGTTTCTGCGGAAGCGCCGCTCAGATTCC AAAGATTCCATAGCTTTAATGGTAGGCTTTCTGGTTCTTCCAGAAGTGCG 5900 CGCAGCTGACGTAGTGGTTGAGTAGCTGGCAGTTGGGGATCCTGGGCCCT CATTGGAACCATCAAGACCAAATTTGTTTCCATACATATCAGCATGGTAT

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Application No.: To be Assigned
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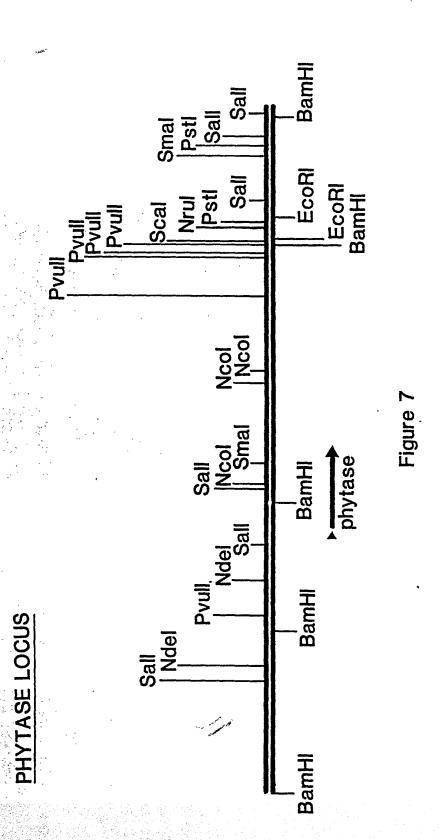
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— Figure 6 (sheet 7 of 7)

TCAAAAGGAAAACTTTCGCCGTACGGAGTACTGCGTTCGATTCCGGGTGT ATCCAAGTCGTATCCAGACATGGTGTCGAATTCAGCCTTGCTGTCAAGAG CAGGGGTACTTTCAATGCTGTCAGCAACCACGCGGCCAAAGGGCGTCTTC GGGAAGAGGTGTTTCAAGAGAGCGTCATCCACGGCCTGGCTTGCGGC GTTGATTGCAGACTTTCGAGTAGATCGCTGAGGTCGCGAACTGGTTCGAG TAGCAACCTGTGAATTGGCAGCCTTGTGACTGCTTCGATTCACTGCAGAG ACGGAGTAGACTGCACTGATTTGGAATTCTGAGTCGCAGCCATTCTGGAT TTGCGTTCGGCGCGACGAGATCTCGCAGTCGTGGTACGAGGAGTAGAGCG AGGCTGCGTAGCAGTGTTGCAAGCTTGGTGCTAGCCTCCTGGGCTTCAGC AGCTTCAGCAGTGGTGGCAGACGCAGCAGAATTAGCGGAGCTTTATCGGC 6500 TTTGCCGCTCTGAGCGTTGGGAGTAGAAGTGAGAGAAGAGGTAGAGTCCA CGGAAGAGTCTTCTCGCTGTTCTCAAAGCCGTTCAGCTTTGCTGGCATA 6600 GACTTACGCGTCTTGCGGCTGTTGGAAGCGGAAGAGTTCATGGCGGGAGA GGAGACGTTAGAAGTAGACATGGTGGGGTTTGTTGACGGGTTTTGAGTAA 6700· CAAGAGACTTGCGTCGATCTTTGAGTGTTCTTGACAGAAAGTTATGCAAC GTCGAC 6756

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SUBSTITUTE SHEET

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#### Figure 8 (sheet 1 of 2)

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CTCCGGACTGGCAGTCCCCGCCTCGAGAAATCAATCCAGTTGCGATACGG S G L A V P A S R N Q S S C D T -1 +1 10	100
TCGATCAGGGGTATCAATGCTTCTCCGAGACTTCGCATCTTTGGGGTCAA V D Q G Y Q C F S E T S H L W G Q	
TACGCACCGTTCTTCTCTCTGGCAAACGAATCGGTCATCTCCCCTGAGGT YAPFFSLANESVISPEV 30 ' 40	200
GCCCGCCGGATCAGAGACTUTEGCTCAGGTCCTCTCCCGTCATGGAG PAGRVTFAQVLSRHG 50	
CGCGGTATCCGACCGACTCCAAGGGCAAGAAATACTCCGCTCTCATTGAG A R Y P T D S K G K K Y S A L I E , 70	300
GAGATCCAGCAGAACGCGACCACCTTTGACGGAAAATATGCCTTCCTGAA E I Q Q N A T T F D G K Y A F L K 80 ' 90	
GACATACAACTACAGCTTGGGTGCAGATGACCTGACTCCCTTCGGAGAAC TYNYSLGADDLTPFGE ' 100 ' 110	400
AGGAGCTAGTCAACTCCGGCATCAAGTTCTACCAGCGGTACGAATCGCTC Q E L V N S G I K F Y Q R Y E S L ' 120 '	
ACAAGGAACATCGTTCCATTCATCCGATCCTCTGGCTCCAGCCGCGTGAT TRNIVPFIRSSGSSRVI 130 ' 140	500
CGCCTCCGGCAAGAATTCATCGAGGGCTTCCAGAGCACCAAGCTGAAGG ASGKKFIEGFQSTKLK 150 ' 160	
ATCCTCGTGCCCAGCCCGGCCAATCGTCGCCCAAGATCGACGTGGTCATT DPRAQPGQSSPKIDVVI 170	600
TCCGAGGCCAGCTCATCCAACAACACTCTCGACCCAGGCACCTGCACTGT SEASSNNTLDPGTCTV 180 190	
CTTCGAAGACAGCGAATTGGCCGATACCGTCGAAGCCAATTTCACCGCCA F E D S E L A D T V E A N F T A	700

200

T.

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## Figure 8 (sheet 2 of 2)

CGTTCGTCCCTCCATTCGTCAACGTCTGGAGAACGACCTGTCCGGTGTG
T F V P S I R Q R L E N D L S G V
220

ACTCTCACAGACACAGAAGTGACCTACCTCATGGACATGTGCTCCTTCGA 800
T L T D T E V T Y L M D M C S F D
230 / 240

CACCATCTCCACCAGCACCGTCGACACCAAGCTGTCCCCCTTCTGTGACC
TISTSTVDTKLSPFCD
250
260

TGTTCACCCATGACGATGGATCAAC ACMACGAGTCCTTGAAA 900 L F T H D E W I N Y D Y L & S L K ' 270 '

AACTATTACGGCCATGGTGCAGGTAACCCGCTCGGCCCGACCCAGGGCGT

KYYGHGAGNPLGPTQGV
280 ' 290

CGGCTACGCTAACGAGCTCATCGCCCGTCTGACCCACTCGCCTGTCCACG

G Y A N E L I A R L T H S P V H

' 300 ' 310

ATGACACCAGTTCCAACCACACTTTGGACTCGAGCCCGGCTACCTTTCCG
D D T S S N H T L D S S P A T F P

320 '

CATTCTCTTTAGGTCTGTAGAACGGCACTAAGCCGCTATCTACCA
I L F A L G L Y N G T K P L S T
350 , 360

CGACCGTGGAGATATCACCCAGACAGATGGATTCTCGTCTGCTTGGACG 1200 T T V E N I T Q T D G F S S A W T 370 '

GTTCCGTTTGCTTCGCGTTTGTACGTCGAGATGATGCAGTGTCAGGCGGA
VPFASRLYVEMMQCQAE
380
, 390

GCAGGAGCCGCTGGTCTTGGTTAATGATCGCGTTGTCCCGCTGC 1300 Q E P L V R V L V N D R V V P L 400 ' 410

ATGGGTGTCCGGTTGATGCTTTGGGGAGATGTACCCGGGATAGCTTTGTG H G C P V D A L G R C T R D S F V 420

TTAG 1404

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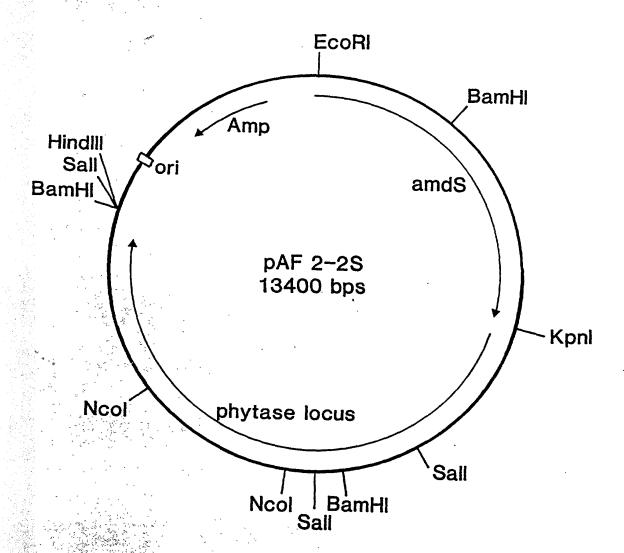


Figure 9

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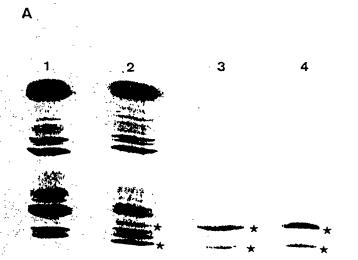


Figure 10a

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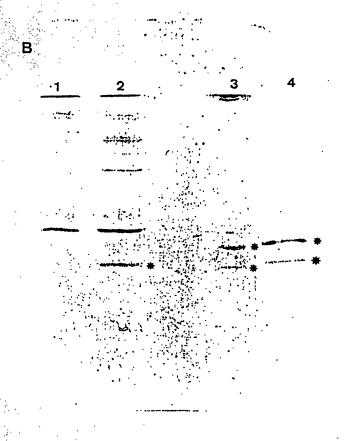


Figure 10b

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Figure 11a

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Figure 11b

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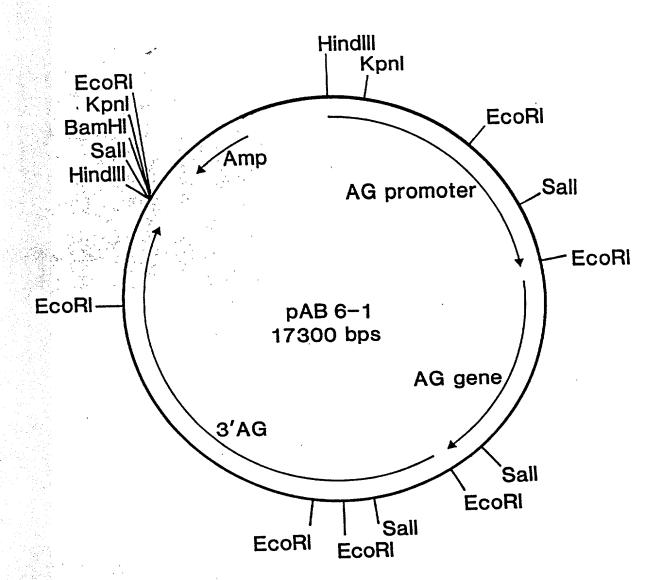


Figure 12

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## AG/PHYTASE GENE FUSIONS BY PCR

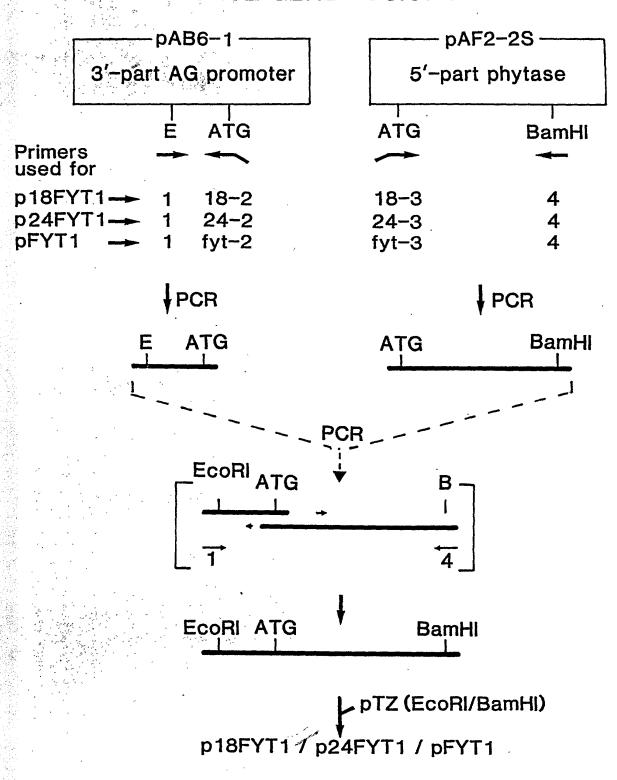


Figure 13

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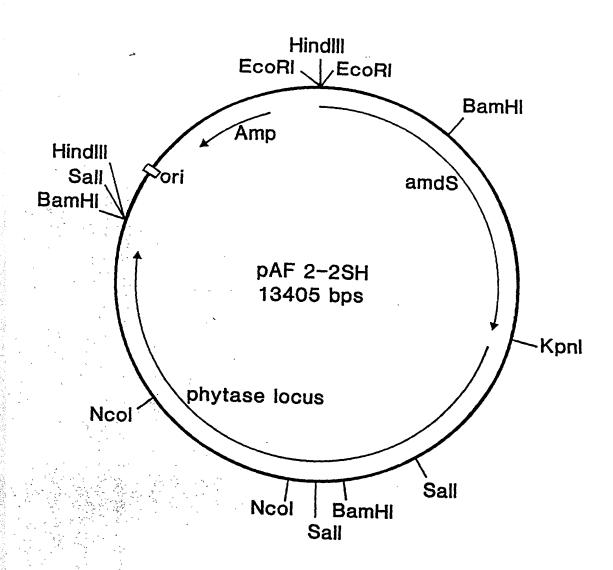


Figure 14

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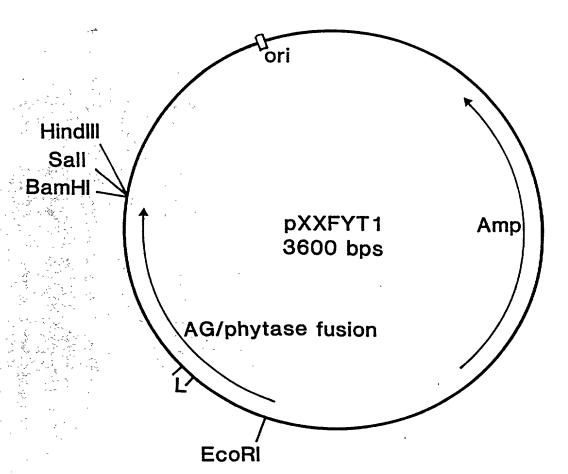


Figure 15a

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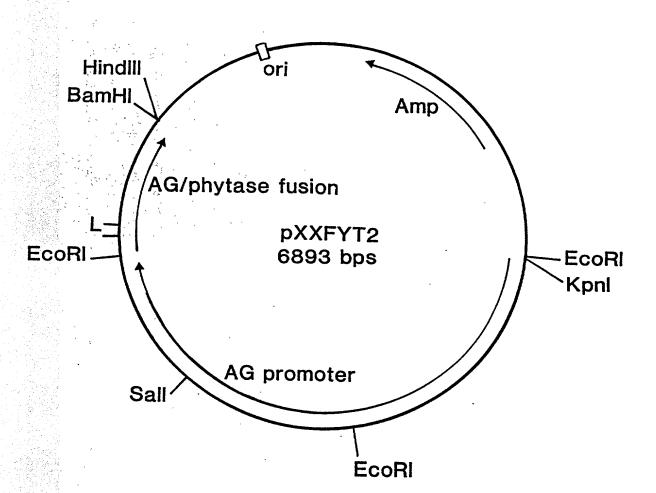


Figure 15b

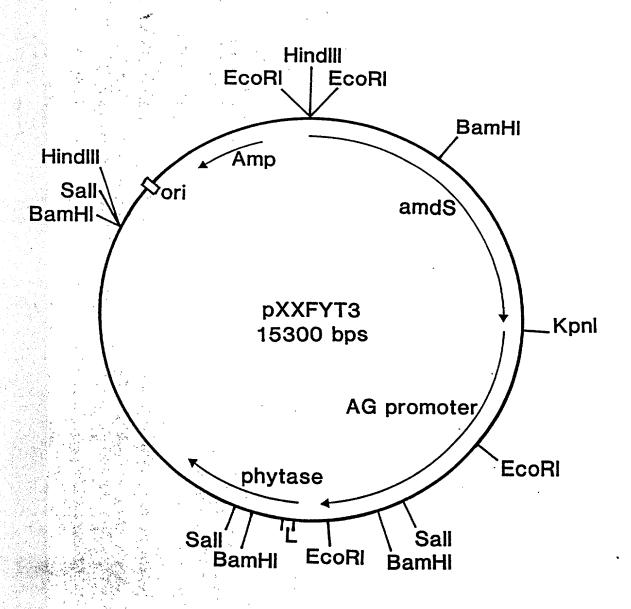


Figure 15c

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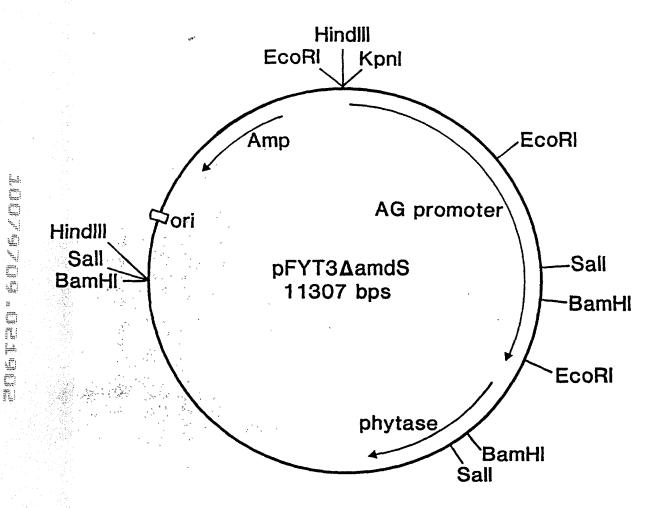


Figure 16

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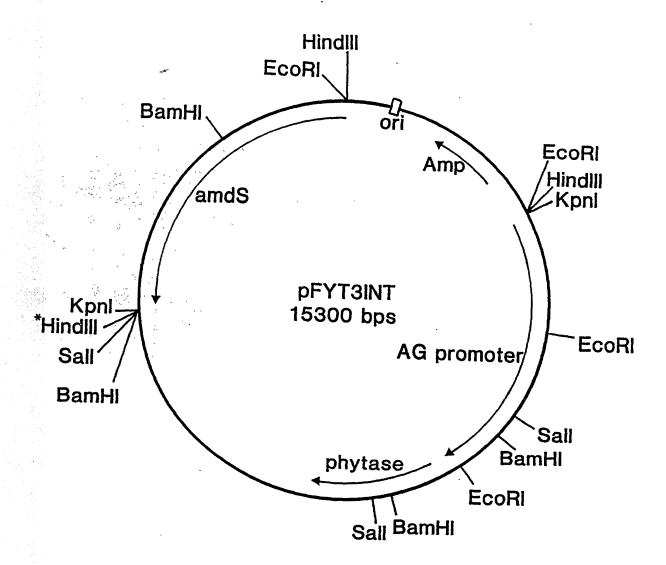


Figure 17

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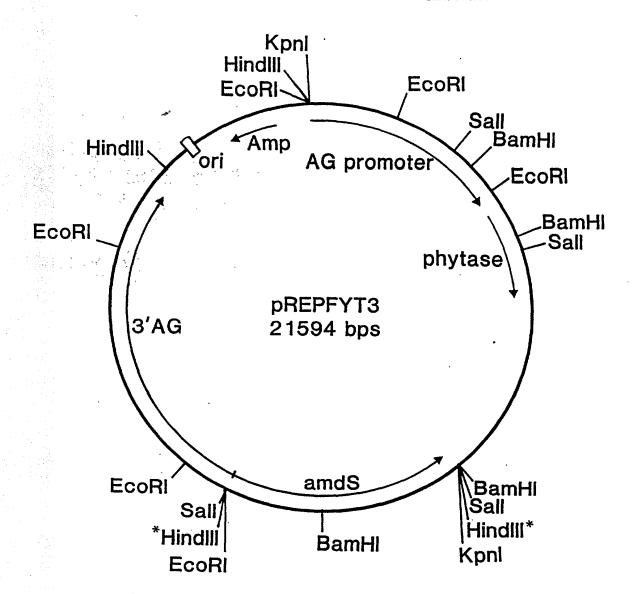


Figure 18

WO 91/05053

Title: CLONING AND EXPRESSION OF MICROBIAL PHYTASE Inventor: Robert VAN GORCOM et al.

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8 9 10 1 1 5 6 7 Kb Mail oil

Figure 19a

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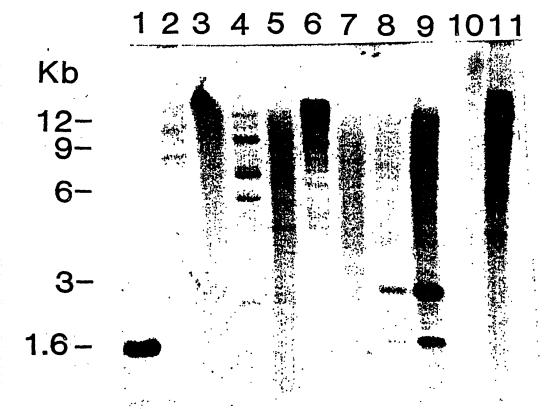


Figure 19b